EVALUATION OF SOME METHODS FOR CONTROLLING THE BOVINE ESTROUS CYCLE

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Introduction

This paper will deal primarily with the evaluation of various methods to control the estrous cycle in cattle. Consideration will be given to four methods of altering the normal estrual cycle. (A) The inhibition of ovulation as a means of altering the estrual cycle appears to be receiving most attention in recent years. The bulk of this paper will deal with ovulation inhibition by progestogens; thus, it would seem appropriate to give a brief evaluation of the other three methods.

(B) Ovulation induction by various means has been so thoroughly studied in the last 25 to 30 years that no additional comments are really necessary at this time. Induction of ovulation during the luteal phase of the estrous cycle has not been accompanied by production of fertilizable ova (Hansel, 5). Few new data have been added to change previous concepts. The use of hypothalamic factors which cause release of endogenous pituitary hormones represents a potential new approach with many intriguing possibilities. Additional information is needed in this area for adequate evaluation.

(C) Inducing or delaying regression of the corpus luteum also offers an interesting approach to estrous cycle control. The best model for regression would be manual removal of the corpus luteum. This procedure has obvious practical shortcomings which have been discussed before (Hansel, 5). Also, the effect of oxytocin during a specific period of corpus luteum formation in the bovine could be included here as an example of an agent altering luteal development. We need to find an agent which is effective at all stages of the estrual cycle before this method can be seriously considered.

(D) Another potential method of controlling the estrous cycle would be to alter the developmental pattern of the heifer so that ovulation would no longer occur spontaneously. A condition sometimes termed "constant estrus" has been produced in female rats by the administration of androgens within the first 5 days after birth (Barraclough and Gorski, 2). The induction of ovulation in a predictable manner could conceivably result in synchronization of ovulation. Our knowledge is inadequate in this area and many questions need to be answered before this approach can be fairly evaluated. Newborn calves and pigs have been injected with androgens within 48 hours after birth (Hansel, 10; Zimbelman, 11), but without results like those obtained from studies of rats.

The balance of this paper will relate some data on ovulation inhibition in cattle with the goal of emphasizing certain guidelines to be used in evaluation of this method to control the estrous cycle.

Experimental Methods

Studies involving dairy heifers were begun only after determining that the heifers were sexually mature and experiencing estrous cycles of normal length. Twice daily observations for estrus and regular rectal palpation of the genital tract were used to determine the influence of treatment. Data on heifers were usually collected under two general procedures: (1) Individual feeding of a compound to dairy heifers for 16 days beginning at the 15th day of the estrous cycle. (2) Group feeding of heifers beginning on the same day, with animals in various stages of the estrous cycle. Oral administration of the progestogens was accomplished by feeding the compound in 1 to 4 pounds of grain per animal daily either at single or divided feedings. Single injections of MAP were made into the ear in volumes up to 5 ml. per ear.

Studies in post-partum cows involved placing cows in their proper group each week at 2 intervals from calving, either 9 to 15 or 16 to 22 days. Animals were randomly assigned to the various experimental groups. Only cows nursing a calf throughout the study were included in the results. The cows were fed 4 pounds of grain daily during the feeding period of 10 or 17 days. After the feeding period, the cows were put to pasture with two bulls. Observations for estrus were made twice daily throughout and after the feeding period until the cows were diagnosed pregnant. Rectal palpations were performed weekly to determine ovarian condition and for pregnancy diagnosis.

Statistical analyses were performed using standard tests described in statistical texts. More detailed descriptions of methods used have been published previously (Zimbelman, 9).

Results and Discussion

Minimal Effective Dosages for Some Compounds

In an earlier publication, dosage titration of medroxyprogesterone acetate (MAP)[1] in heifers was reported (Zimbelman, 9). The

[1] Repromix, ® The Upjohn Company.

minimal effective oral dose was shown to be on the order of 180 mg. daily. Additional studies by other workers and field trials have confirmed that this dosage is effective in beef heifers and cows. Another compound of interest is chlormadinone acetate (CAP) and the minimal effective dose of CAP would appear to be on the order of 5 to 10 mg. daily based on other reports (VanBlake et al., 7; Wagner et al., 8).

Degree of Synchronization Following Inhibition of Ovulation

The degree of synchronization following MAP has also been described in numerous reports. Our data indicated that 95% of the beef and dairy heifers observed in estrus were observed on the second, third, or fourth days after last feeding of MAP at doses in the range of 120 to 400 mg. daily (Zimbelman, 9). Longer intervals from last feeding to first estrus following CAP have been reported (VanBlake et al., 7). This would seem to indicate that either CAP is being used at doses considerably greater than minimal or that the compound acts differently. The answer can come only from simultaneous comparisons at a wide range of doses, some of which are too low to inhibit ovulation.

Conception Rates Following Estrus Synchronization With MAP

The conception rate of animals sychronized with MAP varied from 26 to 60% at the synchronized estrus (S1) in various beef heifer trials (table 1). A consistently high conception rate was noted at the second cycle (S2) of synchronized heifers. A similar trend was found in studies in beef cows over a 2-year period (table 2). The conception rate after two services (S1 + S2) remained quite high regardless of that encountered at the synchronized estrus. Since synchronization with MAP allowed most animals the opportunity to be bred twice in a 25- to 26-day period, the conception rate within the first 26-day period (S26) after last feeding of MAP was calculated to be 77% of the treated animals. The difference between 77% conceiving within 26 days and 89% conceiving with two services was due to two reasons: (1) Failures to detect estrus and, thus, to reinseminate at the second cycle were responsible for about two-thirds of the difference, and (2) in about one-third of the cycles in question, the second ovulations were later than 26 days after last feeding. The fact that conception rates seem to vary markedly from trial to trial conducted in similar fashion should be given consideration by each person planning research on estrous synchronization. Any comparisons of types of animals studied, frequency of feeding, dosage effects, etc., should be made simultaneously. Any deviation from completely contemporaneous comparisons makes the results subject to question.

Effect of MAP on Post-Partum Intervals

The effect of MAP on post-partum intervals in cows was given attention because of its unique importance to successful synchronization. Many cows that are candidates for estrous synchronization may not have returned to normal estrous cycle patterns. Data are available from two trials, one in 1962 with first-calf heifers and one in 1963 with essentially the same group of animals after second calving. The results of the 1962 study were reported earlier (Zimbelman, 9), but are repeated for comparison. The number of animals involved and designs of the trials in 1962 and 1963 are shown in table 3. Basically, the study involved placing cows on trial once each week beginning at two intervals from calving, either 9 to 15 or 16 to 22 days, and feeding for 10 or 17 days. Table 4 shows the average intervals from calving to first post-treatment ovulation and to conception. There were no statistically significant differences in these intervals. However, the relatively short intervals of about 37 and 33 days for cows studied at the early interval from calving and the tendency for treated animals to have shorter intervals to ovulation than control animals are noteworthy. Some untreated cows appeared to have ovulated during treatment, but the average contains only data for the first post-treatment ovulation (table 6).

It should also be stated that we have concluded that rectal palpations of post-partum beef cows do not appear to be nearly as reliable as in cycling dairy heifers. The greatest aid to our study was having fertile bulls present for estrus detection and breeding. A combination of accurate pregnancy diagnosis at 30 to 40 days of gestation, of estrus detection records, and rechecking of weekly ovarian measurements usually allowed us to establish the day of ovulation rather closely. However, any single criterion alone was inadequate.

The average interval from last MAP feeding to next ovulation for treated cows of 4.3 days in 1962 was significantly shorter ($P \cong .05$) than that for the control cows of 8.2 days (table 5). In 1963, the average of 5.3 days for 51 treated cows was significantly shorter ($P < .05$) than the average of 8.9 days for 17 control cows. In addition, the variation in intervals to first post-treatment ovulation was also reduced by treatment in both years. Both the reduction in average interval and in variance are characteristic of a synchronized estrus.

In table 6 the distributions of first post-treatment ovulation with respect to last feeding are shown. In the 2 years, 79% of 73 treated cows ovulated from 2 to 6 days after last feeding compared to 34% of 35 control cows. The control groups had more cows with both shorter and longer intervals. The distributions were significantly different when tested by Chi-Square ($P < .005$). Seven of the 15 control cows in estrus from 7 to 20 days after last feeding had apparently ovulated during the feeding period.

The conception rates for both groups of control cows for 1962 were pooled (table 7). In 1962, the conception rate at first cycle was similar for both treated and control cows, but it was somewhat lower in 1963 for treated cows. These conception rates are based on the assumption that each new ovulation was accompanied by a mating whether or not the mating was observed. These data show that variations in conception rate at the synchronized estrus occur with natural mating as well as with artificial insemination. The conception rate at second service was uniformly high in 1963; thus, the percentage conceiving at two cycles was quite similar in both years between treated and control groups. A higher proportion of treated cows (81% - both years) had conceived by 26 days after last MAP feeding than of control cows (71% - both years). In light of present recommendations, it also seems significant to point out that 79% of all cows had conceived by 59 days post-partum (table 8). This finding would seem to call for a reevaluation of the recommendation that cows not be bred until 60 days post-partum. Of course, it could be argued that the cows used in these studies are not typical of range beef cows. However, it might be fair to assume that these cows at least represent some sort of goal which might be obtained in the efficiency of beef cows if they were receiving proper management and nutrition. These data should not be interpreted as denying that some beef cows under other circumstances may have intervals to first ovulation of 90 days or more.

The data in table 9 illustrate that the interval from calving to conception of about 47 days was confirmed by the average calving interval of 329 days. The average gestation length of 282 days is within the normal range for this breed and is similar to the figure for the previous year for the same animals bred by artificial insemination (Zimbelman, 9). In summary, beef cows were synchronized by beginning treatment with 180 mg. MAP prior to the time that normal ovulation occurred. Additional studies need to be made in cows with longer intervals to ovulation than those of the untreated cows of these studies (that is, 37 days). Foote and Hunter (4) have reported similar findings in a study of the effect of daily injections of progesterone in post-partum beef cows. These workers pointed out that the form of progesterone administration seems important. Another study with a progesterone suspension administered as a single injection delayed rather than shortened the interval to first ovulation (Foote et al., 3). Any oral progestogen to be used in synchronizing estrus in range beef cows should be studied to determine its effect on the interval to first post-partum ovulation. It would seem justified to assume that different results might be obtained from different compounds or from different doses of the same compounds.

Effects of Single Subcutaneous Injections of MAP

In table 10 some data from two trials in which beef heifers received a single subcutaneous injection of MAP in the ear are summarized. In trial A, at 126 days after injection the heifers were slaughtered and the MAP was removed from the ear for chemical analysis. The mean amount of MAP recovered was 21 mg. from 11 heifers receiving 100 mg. and 76 mg. from 13 heifers that had received 500 mg. The average amount absorbed daily was 0.62 and 3.37 mg. for these two doses. Additional data to be presented later will show that the compound was still physiologically active at this time in some heifers (table 11). There were 20 heifers per group in trial B; 5 of each group were slaughtered in four different times from injection varying from 106 to 117 days. The average amount absorbed daily from 100 mg. injection in trial B was only 0.48 mg. daily. Thus, we can conclude that much less than 180 mg. daily is required if the compound is administered in an appropriate manner. Daily injections of 5 mg. intravenously have also proven adequate to inhibit ovulation (Zimbelman, 11). These data demonstrate that MAP is a very insoluble material and would seem to make daily intramuscular or subcutaneous administration a poor means of comparing potency.

The summary of reproductive performance during these studies is shown in table 11. Only one heifer which had received 500 mg. had ovulated at 126 days after injection. About 65% of the heifers receiving 100 mg. MAP begun to ovulate by 106 or more days after injection, as indicated by the presence of new corpora lutea at slaugher. The term "old corpora lutea" refers to those judged to be of a recent ovulation, but earlier than the one responsible for the corpora lutea termed "new." The increased incidence of old corpora lutea in trial B represented less complete inhibition of ovulation by 100 mg. than in trial A. Thus, the effect of single injection varied considerable between heifers within a given trial. Some heifers receiving 100 mg. had not ovulated recently while others had one or two sets of recent corpora lutea. The incidence of palpable corpora lutea also indicated that the effect of 100 mg. varied widely between trials as well, much greater effects being achieved in trial A than in trial B. Essentially no animals ovulated in trial A for 10 to 12 weeks after injection, while some animals in trial B never stopped ovulating. Thus, the chances of achieving synchronization of estrous cycles following a single injection seem rather remote. Rectal palpation at intervals of about every 2 weeks revealed an increased incidence and size of detectable follicles due to MAP treatment.

Characterization of Follicular Activity During Progestogen Influence

The follicular fluid weights of heifers receiving single injections of MAP are shown in table 12. These data were obtained by weighing the ovary intact, and again after slicing at 5 mm. intervals. In trial A, there was a significant increase in follicular fluid due to treatment. That there was more follicular fluid at 500 mg. appears to be due to the greater number of follicles since more heifers from the 100 mg. group had ovulated recently. The failure to find a significant increase in follicular fluid in trial B would appear to be due to the return to normal cyclic ovulation by most treated heifers by the end of trial B. Follicular fluid weights were also increased about threefold (1.33 vs. 4.01 gm.) during daily oral treatment with a progestogen at a dosage adequate to inhibit ovulation (Zimbelman, 11).

Evidences of Estrogenic Influence

It has been pointed out before that vulvar swelling, increased uterine tone, and frequent discharges of mucus occur in heifers during the period of ovulation inhibition with an oral progestogen (Zimbelman, 9). These signs are typical of an estrogenic influence. Cervical mucus smears from heifers which received a progestogen orally beginning at various stages of the estrual cycle were studied in accord with the procedures of Alliston *et al.*, (1). Briefly, ratings of 1 and 2 represent little or no fern pattern, rating of 3 and 4 represent typical patterns over less than the complete smear, while ratings of 5 and 6 represent typical patterns over the entire smear. These smears were classified as coming from heifers in which the corpus luteum was or was not regressed. Regression was defined as reduction of 4 mm. in estimated size or disappearance of the corpus luteum found on previous palpation. After regression of the corpus luteum, 72% of the smears had a typical fern pattern as compared to only 17% when the mature corpus luteum was present (table 13). This difference was statistically significant ($P < .005$). Thus, oral progestogens may prevent ovulation as effectively as does the corpus luteum, but there appeared to be a definite estrogenic influence that was not detected in the luteal phase. The means by which the corpus luteum inhibits ovulation needs to be clarified. After regression of the corpus luteum, there was no difficulty in penetration of the cervix; this also is more typical of the estrual than of the luteal phase. The evidence seems to indicate a high degree of follicular stimulation both in terms of follicle size and of estrogenicity. If there is any basis for combination of progestogens and estrogens for estrous cycle control in cattle, it would not seem to be due to an estrogen deficiency under usual conditions.

Agents Which Will Induce Ovulation

One partial test of the mechanism of ovulation inhibition could be made by determining agents which will overcome the progestogen-induced inhibition. To carry out studies of this nature, heifers received a progestogen orally from day 15 after estrus until one week after injection of a possible ovulation inducer. Preliminary studies revealed that several estrogens were effective, the results varying with the route of administration and the vehicle used. Several gonadotropins appeared to be effective. Agents which failed to induce ovulation were: oxytocin (200 I.U.), amphetamine (300 mg.), and neostigmine (50 mg.). Ovulation was induced in 16 heifers each with an intravenous injection of either: 10 mg. estradiol cyclopentylpropionate, 5,000 I. U. of Chorionic Gonadotropin,[2] or 50 mg. of pituitary luteinizing hormone.[3] Based on gross appearance, the corpora lutea corresponding to days 1 and 4 of the estrous cycle seemed normal. However, at stages corresponding to days 7 and 10 of the estrous cycle, many induced corpora lutea were beginning to regress. This is a finding not too dissimilar from the effects of oxytocin injected early in the estrual cycle (Simmons and Hansel, 6). In light of recent findings on factors affecting the life of the bovine corpus luteum, this effect may be interpreted as an indication of an LH deficiency which is responsible for failure of the corpus luteum to persist the usual length of time (Simmons and Hansel, 6). These findings would tend to discourage synchronization techniques which involve induction of ovulation during a period of continued progestogen treatment or during persistence of progestogen levels.

General Discussion

The increased follicular activity and cervical mucus smear ratings suggest very little alteration of the FSH (follicle stimulating) levels in treated heifers. According to one concept of stimuli needed for estrogen production, it could also be speculated that there must be some LH influence on the ovary as well as FSH influence. In recent years, several workers have concluded that two

[2] Chorionic Gonadotropin (Upjohn).

[3] P.L.H. (Armour).

hypothalamic centers exist for release of pituitary LH (Barraclough and Gorski, 2). One center is postulated to control tonic release of small amounts of LH and the other is regarded as controlling the cyclic release of large amounts of LH as needed for ovulation. Thus, one interpretation of the mechanism of oral progestogen inhibition is that only the cyclic release of pituitary LH in the amounts needed for ovulation has been inhibited. This would allow normal FSH levels and levels of tonic LH adequate to produce the large follicles and the apparent resultant estrogenic influence.

Of the presently known methods of altering the estrous cycle of the cow, the most promising at present appears to involve the inhibition of ovulation. Of the various possible means to inhibit ovulation, the use of progestational steroids appears to be receiving the most attention. Some of the presently used progestogens are very difficult to characterize. The relative potency by a common route varies markedly with species and the potency within a species varies markedly by route of administration. One cannot characterize hormones, he can only describe the effects of certain dosages of hormones by a given route. Thus, it seems necessary to adequately determine the physiologically effective dose by the intended route of administration prior to comparing two hormones or compounds.

Summary and Conclusions

Various methods of altering the estrual cycle of the cow were discussed. Ovulation inhibition was selected for further discussion and data relating to the use of progestogens for this purpose were discussed. The conception rate at the synchronized estrus varied from 26 to 75% in various trials, but was consistently high at the second cycle. This type of variation requires that comparisons of compounds or procedures used be made completely contemporaneous. Ovulation was synchronized in post-partum beef cows placed on treatment prior to the resumption of normal estrual cycles. Additional work is needed on other compounds and with cows that require longer intervals to first post-partum estrual cycle. A single subcutaneous injection of 100 to 500 mg. MAP prevented ovulation for more than 106 days in many heifers. The residual MAP was removed and analyzed at slaughter and as much as 46% remained after 106 days. Follicular activity was greater during the period in which ovulation was inhibited than during the normal estrual cycle. Both the size and incidence of palpable follicles increased following a single injection of MAP. Cervical mucus smears showed typical fern patterns suggesting that the increased follicle size during oral progestogen treatment was associated with increased estrogen production. These findings should be considered in the interpretation of side effects and after effects. The progestogen block of ovulation can be overcome by administration of estrogens in the proper vehicles and route and by various gonadotropins (pituitary luteinizing hormone and Chorionic Gonadotropin). Corpus luteum formation and persistence were not always of normal proportions, however. Such findings tend to discourage synchronization techniques which induce ovulation in cattle during a period of continued progestogen influence. Discussion supports the concept that ovulation inhibition with progestogens was accomplished by inhibition of the mechanism causing cyclic release of pituitary ovulating hormone.

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Table 1.--Conception rates of heifers after synchronization with MAP

Number of Animals:	Dairy Heifers	Beef Heifers - 1961[b] B1	B2	B3
Treated	46	36	35	24
Synchronized	45	35	35	19
Untreated	8	-	-	12
Conception Rates (Percent):				
S1[a]	58%	60%	26%	37%
S2	62%	87%	85%	83%
S1 + S2	85%	94%	89%	92%
S26	78%	67%	77%	75%
U1	75%	-	-	67%

[a] S1 = First service of treated (synchronized) animals.
S2 = Second service of treated animals.
S1 + S2 = Treated animals conceiving with two services.
S26 = Treated animals conceiving within 26 days after last feeding.
U1 = First service of untreated (control) animals.

[b] Data on 3 beef heifer trials (B1, B2, B3) combined in table 2.

Table 2.--Conception rates of heifers and cows after synchronization with MAP

Number of Animals:	Dairy Heifers	Dairy Heifers	Beef Cows[b] 1962	1963	Total
Treated	46	95	22	51	214
Synchronized	45	89	20	42	196
Untreated	8	12	18	17	55
Conception Rates (Percent):					
S1[a]	58%	42%	75%	39%	47%
S2	62%	85%	50%	81%	78%
S1 + S2	85%	91%	86%	88%	89%
S26	78%	76%	86%	78%	77%
U1	75%	67%	78%	59%	69%

[a] See table 1 for explanation of abbreviations.

[b] All beef cows were bred by natural service.

Table 3.--Design of studies to determine influence of MAP on post-partum reproductive performance of beef cows

	Controls		Treated 180 mg. MAP			Year
Days PP[a]	9-15	16-22	9-15	16-22	16-22	total
Days Fed	17	17	17	10	17	-
			Number of Cows			
Year						
1962	9	9	12	0	10	40
1963	17	0	17	17	17	68
Total	26	9	29	17	27	
Pooled Total	35			73		108

[a] Days PP = Days from calving to first grain feeding.

Table 4.--Average intervals from calving as influenced by MAP

	Controls		Treated 180 mg. MAP		
Days PP	9-15	16-22	9-15	16-22	16-22
Days Fed	17	17	17	10	17
Last Feeding[a]	27.7	35.2	27.9	27.0	35.2
	Days Post-Partum to: First Post-Treatment Ovulation				
Year					
1962	36.6	43.6	32.5	-	39.9
1963	35.9	-	32.6	32.8	40.0
	Conception Rates (Percent)				
1962	39.1	59.7	43.9	-	41.6
1963	50.3	-	51.3	51.5	52.5

[a] The average interval from calving to last feeding was obtained for the two years.

Table 5.--Average intervals and variation to first post-treatment ovulation

	Controls			Treated 180 mg. MAP			
Days PP	9-15	16-22	Pooled	9-15	16-22	16-22	Pooled
Days Fed	17	17	-	17	10	17	-
	Days From Last Feeding: Mean						
Year							
1962	8.0	8.4	8.2	4.2	-	4.5	4.3[a]
1963	8.9	-	8.9	4.9	5.8	5.1	5.3[b]
	Standard Deviation						
1962	6.3	9.5	7.8	1.7[c]	-	3.5[c]	2.6[d]
1963	7.8	-	7.8	4.7	6.5	5.1	5.4[b]

[a] Differs from pooled control (.06>P>.05).
[b] Differs from pooled controls (P<.05).
[c] Differs from respective control (P<.01).
[d] Differs from pooled controls (P<.001).

Table 6.--Distribution of first post-treatment ovulation after last MAP feeding

Days After Last Feeding	Percent of Cows: Controls	Treated
0 to 1	17%	3%
2 to 6	34%	79%
7 to 20	43%[a]	14%
21 or >	6%	4%
N	35	73

Chi-Square Value: 22.75 (P<.005)

[a] Of 15 cows in this group, 7 had apparently ovulated during the feeding period.

Table 7.--Conception rates of post-partum cows

	Controls	Treated 180 mg. MAP			
Days PP	-	9-15	16-22	16-22	Pooled
Days Fed	17	17	10	17	-
		First Cycle			
Year					
1962	78%	58%	-	90%	73%
1963	59%	35%	29%	53%	39%
		Second Cycle			
1962	50%	40%	-	100%	50%
1963	57%	82%	75%	88%	81%
		Two Cycles			
1962	89%	75%	-	100%	86%
1963	82%	88%	82%	94%	88%
		By Day 26 ALF[a]			
1962	72%	75%	-	100%	86%
1963	71%	76%	71%	88%	78%

[a] Percentage of group pregnant by 26 days after last feeding of MAP.

Table 8.--Distribution of interval from calving to conception

	Cumulative Percent of Cows Conceiving[a]		
Days Post-Partum	Controls	Treated	All Cows
27 to 39	46%	32%	36%
40 to 49	69%	60%	63%
50 to 59	74%	81%	79%
60 or >	100%	99%	99%
N	35	73	108

Chi-Square: 5.67, (P<.10) for comparison of controls and treated.

[a] Based on cows conceiving with 4 or less services.

Table 9.--Intervals between calvings and gestation length

	Controls	Treated	All Cows
Number of Cows	18	20	38
Average Intervals:			
Calving to Conception	49.4	44.5	46.8
Calving to Calving[a]	332.9	325.9	329.2
Gestation length	283.5	281.4	282.4

[a] The number of days from date calved in 1962 to date calved in 1963.

Table 10.--Recovery of residual MAP from ears of injected heifers

Trial	Mg. MAP Injected	MAP Recovered: Range (Mg.)	MAP Recovered: Mean Mg.	MAP Recovered: Mean %	Mg. MAP Absorbed: Total	Mg. MAP Absorbed: Daily
A	100	0 to 43	21	21	79	0.62[a]
	500	18 to 212	76	15	424	3.37
B	50	4 to 36	21	42	29	0.26[b]
	100	21 to 69	46	46	54	0.48

[a] The test period was 126 days for trial A.
[b] Five heifers were killed at each of 106, 110, 113, and 117 days from injection, averaging 111.5 days for the 20 animals.

Table 11.--Summary of ovarian activity following single MAP injections

MAP Injected	No. Heifers	Rectal Palpation: Overall Incidence[a]: Corpora Lutea	Rectal Palpation: Overall Incidence[a]: Follicles	Rectal Palpation: Follicle[b] Size	Slaughter Findings: New C.L.	Slaughter Findings: Old C.L.
-	13	71%	37%	14.0	100%	100%
100	13	7%	84%	16.6	64%	18%
500	13	3%	81%	14.8	8%	0%
(N)[c]		(230)	(230)	(154)		
-	20	78%	38%	14.0	100%	100%
50	20	54%	61%	14.9	100%	95%
100	20	32%	77%	16.3	65%	65%
(N)		(510)	(510)	(298)		

[a] Percent of rectal palpations at which a corpus luteum or follicle was detectable.
[b] The average size (mm.) of follicles as estimated on rectal palpation.
[c] Indicates the number of rectal palpations performed or the number of follicles measured.

Table 12.--Ovarian weights of heifers treated with MAP

Trial	MAP Injected	No. Heifers	Ovarian Weight: Total Intact (Grams)	Ovarian Weight: After Slicing[a] (Grams)	Follicular Fluid Weight[b]: Largest Follicle (Grams)	Follicular Fluid Weight[b]: Total (Grams)
A	-	13	11.34	9.93[c d]	-	1.41
	100	11	14.79	10.85[c]	-	3.94[d]
	500	13	11.77	7.39[d]	-	4.38[d]
		D[e]	3.49	2.81		2.10
B	-	20	13.30	11.34	1.08	1.96
	50	20	14.38	12.30	1.21	2.07
	100	20	14.71	11.74	1.83	2.97
		D[e]	2.63	2.17	.97	1.30

[a] The ovary was sliced at 5 mm. intervals and fluid was blotted.
[b] The follicular fluid weight represents the difference between total ovarian weight and weight after puncture of the largest follicle or after slicing at 5 mm. intervals.
[c d] Mean values bearing the same superscript are not significantly different ($P < .05$).
[e] D = Difference required for statistical significance ($P < .05$).

Table 13.--Cervical mucus smear ratings of dairy heifers receiving oral progestogen for 8 or more days

	During progestogen treatment	
Percent rated as:[a]	Mature corpus luteum	Corpus luteum regressed[b]
1 or 2	83%	28%
3 or 4	17%	47%
5 or 6	0%	25%
N	41	128

Chi-Square 39.78 ($P < .005$)

[a] See text for description of rating procedure.
[b] Regression defined as no palpable corpus luteum or estimated size decreased by 4 mm. or more.